Sophi™ Toxin B Activity Assay RUO

Determination of Toxin B activity in feces is a Sophi™ affinity chip assay that measures real enzymatic activity of Toxin B produced by Peptostreptococcaceae and Clostridiaceae families.

BACKGROUND

An anaerobic, spore-forming, Gram-positive rod, Clostridioides difficile represents a severe pathogen causing intestinal infections with a heterogeneous clinical picture from asymptomatic carriages, mild or moderate diarrhea to life-threatening toxic megacolon colitis. The crucial virulence factor is the production of secreted toxins, including Toxin B. Laboratory diagnostics of C. difficile in patients with suspected infection is based mainly on enzyme immunoassays detecting toxins and glutamate dehydrogenase (GDH) as an antigen, without differentiating between toxigenic and non-toxigenic strains. Another detection option is to use molecular-genetic techniques that detect genes for toxins and GDH without evaluating their clinical manifestation. Because C. difficile can colonize the gastrointestinal tract without symptoms of infection, it is crucial to determine not just the presence of the microorganisms, but also free active toxins. Positive results obtained by PCR can lead to misidentification of a true pathogen, resulting in inappropriate therapy. Negative results, on the other hand, can lead to insufficient treatment because toxins causing gastrointestinal tract infection can be produced by other bacteria, e.g., P. sordellii, many of them without a commercial ELISA assay available. The virulence genes, including those encoding for toxins, can be spread in microbial populations by horizontal gene transfer, which again complicates diagnostics based on determination of the microorganism. The detection of toxins and their activity is therefore crucial to confirm proper diagnosis of C. difficile-related infections and for evaluating its risk and progression.

TEST

The patient's stool sample isolates in reaction buffer (2 uL) are incubated on a **Sophi™** target array with immobilized affinity-tagged substrate protein. The immobilized substrate protein undergoes enzymatic modification due to the presence of Toxin B in the stool isolate. The enzymatic modification takes place at 37 °C in the humidity chamber, preventing evaporation of the droplets on the **Sophi™** target array. After one hour of incubation, the chip is washed 3 times with a washing buffer and 3 times with distilled water to remove any non-specifically bound agents. The wash protocol leaves only modified/unmodified substrate protein on the chip. The MALDI matrix is applied to the dried surface. The toxin presence is confirmed by observing a mass shift that represents addition of glucose to the substrate protein catalyzed by the active Toxin B present in the isolate.

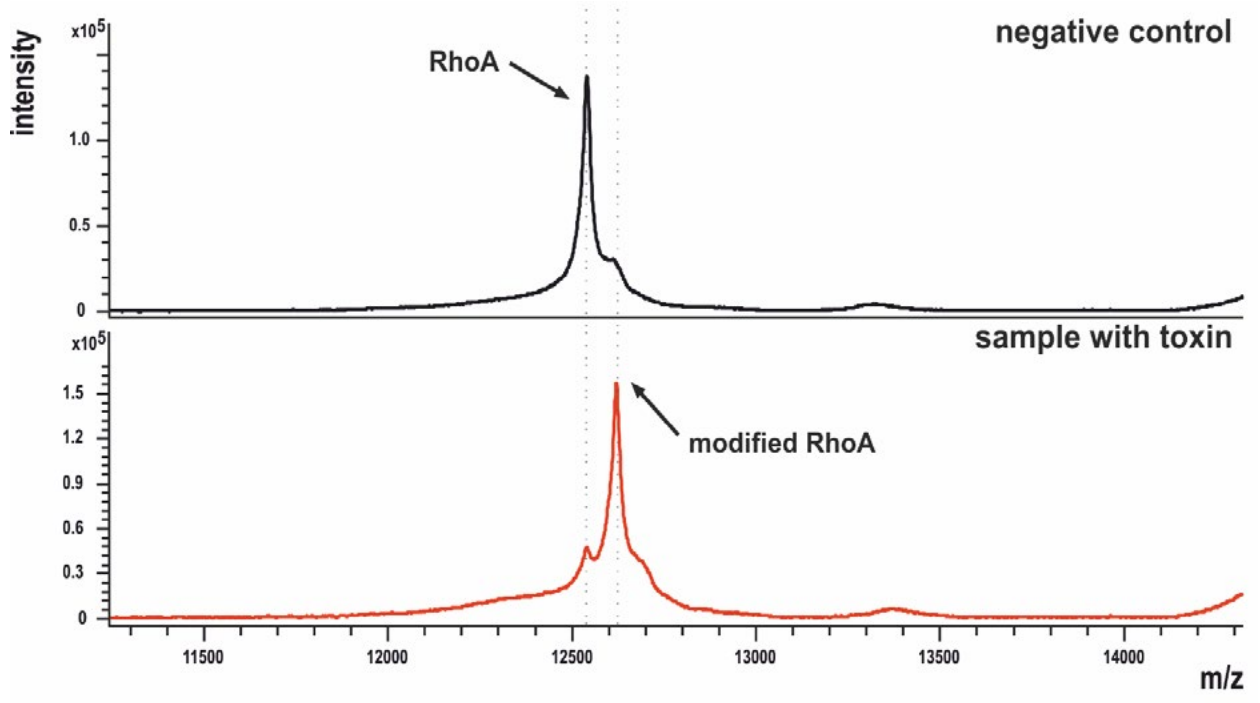
info@massspecmedical.com
www.massspecmedical.com
(under construction as of February 2024)

CLINICAL USE

The determination of Toxin B activity in feces helps assess the severity of the bacterial infection and its potential progression to pseudomembranous colitis, megacolon, and gut perforation. Another motivation for detecting the free active toxin, instead of just the microorganism, is in proper monitoring of modern biological therapies of C. difficile-related infections that are based on neutralization titration of the free toxins with monoclonal antibodies (e.g. Bezlotoxumab).

DATA EXAMPLE

MALDI-MS spectra of Toxin-B substrate (RhoA) obtained by **Sophi**™ assay. Top shows feces extract of a healthy individual, bottom of an infected patient. The mass shift is recognized by the software, evaluated and positive detection is automatically reported.



Detailed information

Dvorak et al.: The rapid detection of procalcitonin in septic serum using immunoaffinity MALDI chips, Clin Proteomics. 2023, 20 (1) 20. doi: 10.1186/s12014-023-09410-3.

info@massspecmedical.com
www.massspecmedical.com
(under construction as of February 2024)